PATENT SPECIFICATION (11) 1407903

1407903

(21) Application No. 13547/73 (22) Filed 21 March 1973
(23) Complete Specification filed 18 Feb. 1974
(44) Complete Specification published 1 Oct. 1975
(51) INT CL² C07H 19/20 A61K 31/675
(52) Index at acceptance
C2P 1L1 2E13 2E15A 2E18B 2E18C 2E19B 2E19C 2E19D 2E19F 2E26A 5A 5B 6 7 9 A

(72) Inventors RALPH HOWE, JOHN ROGER TITTENSOR MICHAEL DEREK EDGE and GILBERT JOSEPH STACEY

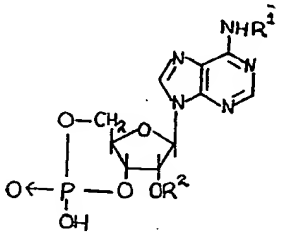
(19)

(54) NUCLEOTIDE DERIVATIVES

(71) We, IMPERIAL CHEMICAL INDUSTRIES LIMITED, Imperial Chemical House, Millbank, London SW1P 3JF, a British Company, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement: —

This invention relates to nucleotide derivatives, and more particularly it relates to novel acylated derivatives of adenosine - 3′,5′ - cyclic monophosphate (cyclic AMP) which possess the property of inhibiting the aggregation of blood platelets.

According to the invention there is provided a 9 - H - adenine - β - D - ribofuranoside - 3′,5′ - cyclic monophosphate derivative (hereinafter referred to as a cyclic AMP derivative) of the formula: —



I

wherein R^1 is an acyl group of the formula —CO . OR^3 or —CO . NHR^4, wherein R^3 is an alkyl radical of 1—8 carbon atoms, a haloalkyl radical of 1—4 carbon atoms, or an alkenyl radical of up to 4 carbon atoms, and R^4 is an alkyl radical of 1—8 carbon atoms, a haloalkyl radical of 1—4 carbon atoms, an alkenyl radical of up to 4 carbon atoms, a phenylalkyl radical of 7—9 carbon atoms, a phenyl radical, a naphthyl radical, or a phenyl radical bearing as a substituent a halogen atom or an alkyl or alkoxy radical of 1—3 carbon atoms; and R^2 is hydrogen or an acyl group of the formula —CO . OR^3, —CO . NHR^4 or —CO . R^5, wherein R^3 and R^4 are as defined above and R^5 is an alkyl radical of 1—4 carbon atoms, and the base addition salts thereof.

In this specification, the compound of formula I wherein R^1 and R^2 are both hydrogen is named cyclic AMP, and the substituent R^1 is said to be in the N^6-position whereas the substituent R^2 is said to be in the $O^{2'}$-position. The compounds of the invention are thus named as N^6 - mono - acyl or N^6,$O^{2'}$ - di - acyl derivatives of cyclic AMP.

An example of a suitable value for R^3 or R^4 when it is an alkyl radical of 1—8 carbon atoms is a methyl, ethyl, n-propyl, n-butyl, isobutyl, n-hexyl, n-heptyl or n-octyl radical, but a preferred value for R^3 or R^4 is an alkyl radical of 3—6 carbon atoms, for example a n-propyl, n-butyl, isobutyl or n-hexyl radical.

An example of a suitable value for R^3 or R^4 when it is a haloalkyl or alkenyl radical of up to 4 carbon atoms is a 2,2,2 - trichloroethyl, 2 - chloroethyl or allyl radical, and an example of a suitable substituent which may be present on a phenyl radical when it is a value for R^4 is an *o-*, *m-* or *p*-chloro substituent, or a *p*-methoxy radical.

An example of a suitable value for R^4 when it is a phenylalkyl radical is a benzyl radical.

A particular value for R^5 is an n-propyl radical.

A base addition salt of the invention may be a pharmaceutically-acceptable base addition salt, for example an alkali metal or alkaline earth metal salt, for example a sodium, potassium or calcium salt. Alternatively, it may be a base addition salt which is primarily used in the manufacture of the compounds of the invention because of the desirable physical properties of that salt, but which is not necessarily pharmaceutically-acceptable. Examples of such salts are a barium salt, or a salt with an organic amine, preferably a tertiary amine, for example trimethylamine, triethylamine, di - isopropylethylamine, pyridine, or an N - alkylmorpholine, for example N - methylmorpholine.

Particularly useful compounds of the inven-

[Price 33p]

tion are those cyclic AMP derivatives of formula I wherein both R^1 and R^2 are acyl groups, that is when R^2 is other than hydrogen. They may be, for example, compounds in which R^1 and R^2 are both acyl groups of the formula —CO . OR^3 or both acyl groups of the formula —CO . NHR^4, or they may be compounds in which R^1 is an acyl group of the formula —CO . R^3 or —CO . NHR^4 and R^2 is a different acyl group of the formula —CO . OR^3, —CO . NHR^4 or —CO . R^5.

Specific cyclic AMP derivatives of the invention are illustrated in the accompanying Examples, and of these $N^6,O^{2'}$ - di - phenylcarbamoyl - c.AMP is particularly preferred, and $N^6,O^{2'}$ - di - isobutoxycarbonyl - c.AMP and $N^6,O^{2'}$ - di -n - butoxycarbonyl - c.AMP are also particularly potent.

The cyclic AMP derivatives of the invention may be prepared by processes which are known for the preparation of analogous acylated derivatives of cyclic AMP. Thus the following processes, in which R^1, R^2, R^3, R^4 and R^5 have the meanings stated above, are provided as further features of the invention:

1) For a compound of formula I wherein R^1 is an acyl group of the formula —CO . OR^3 or —CO . NHR^4 and R^2 is hydrogen or R^1 and R^2 are identical acyl groups of the formula —CO . OR^3 or —CO . NHR^4, reacting cyclic AMP, or a base addition salt thereof, with at least 1.5 molecular equivalents of an acylating agent derived structurally from an acid of the formula R^3O . CO . OH or R^4NH . CO . OH.

If a base addition salt is not used as starting material the reaction is preferably carried out in the presence of a base. Convenient base addition salts are as stated above.

A particularly suitable acylating agent is an acid halide, for example an acid chloride, or an anhydride of the above-mentioned acids, and the base may be a volatile organic base, for example pyridine or di-isopropylethylamine. If two or more molecular equivalents of acylating agent are used, the main product obtained is a compound of formula I in which both R^1 and R^2 are acyl groups, but smaller quantities of compounds of formula I, in which either R^1 or R^2 is an acyl group, are obtained and may be isolated.

2) For a compound of formula I wherein R^1 is an acyl group of the formula —CO . NHR^4, and R^2 is hydrogen, or R^1 and R^2 are identical acyl groups of the formula —CO . NHR^4, reacting cyclic AMP, or a base addition salt thereof, with at least 1.5 molecular equivalents of an isocyanate of the formula $R^4N=C=O$, wherein R^4 is as stated above.

If two or more molecular equivalents of isocyanate are used, the main product is a compound of formula I wherein both R^1 and R^2 are acyl groups and smaller quantities of monoacyl derivatives are obtained.

The reaction is conveniently carried out in a diluent or solvent, for example N,N - dimethylformamide or pyridine, and a convenient base addition salt is as stated above.

3) For a compound of formula I wherein R^2 is hydrogen, hydrolysing the corresponding cyclic AMP derivative of formula I, wherein R^2 is an acyl group, with dilute alkali, for example 0.1N sodium hydroxide or potassium hydroxide solution, for a period of from 15 minutes to 48 hours at ambient temperature, or by hydrolysing under the same conditions a tri-acylated derivative of cyclic AMP, usually obtained as a by-product of process 1) or 2).

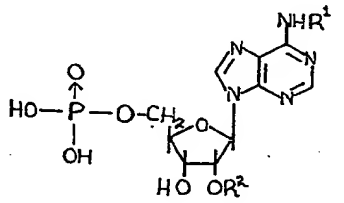
4) For a compound of formula I wherein R^2 is other than hydrogen:

a) reacting the corresponding cyclic AMP derivative of formula I, wherein R^2 is hydrogen, or a base addition salt thereof, with an acylating agent derived structurally from an acid of the formula R^3O . CO . OH, R^4NH . CO . OH or R^5 . CO . OH, or with an isocyanate of the formula R^4 . N=C=O.

or b) reacting the corresponding cyclic AMP derivative of formula I, wherein R^1 is hydrogen and R^2 is an acyl group of the formula —CO . OR^3, —CO . NHR^4 or —CO . R^5, or a base addition salt thereof, with an acylating agent derived structurally from an acid of the formula R^3O . CO . OH or R^4NH . CO . OH, or with an isocyanate of the formula R^4 . N=C=O.

Process 4) is carried out under the same conditions as processes 1) and 2) and is particularly convenient for the preparation of compounds of the invention wherein R^1 and R^2 represent different acyl groups.

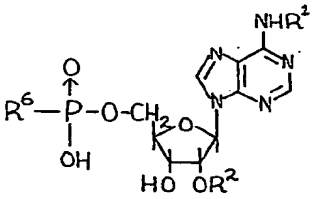
5) Dehydrating an adenosine - 5' - phosphate derivative of the formula: —



II

A suitable dehydrating agent is, for example a carbodiimide, for example, N,N' - dicyclohexylcarbodi - imide or N,N' - di - *p* - tolylcarbodi - imide, and the dehydration may be carried out in a diluent or solvent, for example formamide, dimethylformamide or pyridine. The dehydration is conveniently carried out at a temperature of 80—120°C., and the nucleoside - 5' - phosphate is conveniently used in the form of a salt which is soluble in the reaction medium, for example a 4 - morpholine - N,N' - dicyclohexylcarboxamidinium salt or triethylammonium salt.

6) Reacting a compound of the formula: —



III

wherein R^6 is a radical derived from an acid R^6H which is more acidic than the 3'-hydroxyl radical of the nucleotide of formula III, with a base.

A particularly suitable value for R^6 is a halogen atom, a phenoxy radical optionally bearing one or more nitro, halogen or methylsulphonyl radicals, an alkoxy radical, especially a methoxy radical, bearing a cyano radical or a halogenomethyl radical, or a diaryl phosphate radical. Examples of such suitable values for R^6 are a fluorine atom, a 2,4 - dinitrophenoxy, *p* - nitrophenoxy, 2,4,5 - trichlorophenoxy, *p* - methylsulphonylphenoxy, cyanomethoxy, 2,2,2 - trichloroethoxy or diphenyl phosphate radical.

The reaction is conveniently carried out in a diluent or solvent, for example a polar organic solvent such as dimethylsulphoxide, t-butanol, dimethylformamide. Preferably the diluent or solvent should be anhydrous, and this is particularly important when R^2 stands for an acyl group in order to avoid replacement of such a radical by hydrogen owing to hydrolysis.

The reaction is conveniently carried out at a temperature from −20°C. to 40°C., and a suitable base is, for example, an alkali metal alkoxide, for example sodium or potassium methoxide or t-butoxide, or an alkali metal hydride, for example sodium or potassium hydride.

Processes 5) and 6) are most useful when R^2 is hydrogen.

As stated above, the cyclic AMP derivatives of the invention possess the property of inhibiting the aggregation of blood platelets. This property is demonstrated *in vitro* by adding the test compound to a stirred sample of heparinised human platelet-rich plasma, and measuring the effect of the test compound in delaying or reducing the increase in the light transmission of the sample caused by the addition of collagen or adenosine 5'-diphosphate. In this test, the cyclic AMP derivatives of the invention inhibit the aggregation of blood platelets at concentrations as low as 10^{-3} molar, and the most potent compounds show this effect at concentrations as low as 10^{-5} to 10^{-6} molar. The *in vitro* inhibition of the aggregation of blood platelets is useful in preserving blood for transfusion purposes without impairing its function.

The ability of the cyclic AMP derivatives of the invention to inhibit the aggregation of blood platelets can also be demonstrated *in vivo* using a standard test in rabbits in which the test compound is administered by intravenous injection or infusion to the animal, and a sample of blood is withdrawn and its capacity to resist platelet aggregation induced by collagen is measured by its decreased resistance to flowing through a microscope filter compared with a control sample to which collagen has also been added. In this test, $N^6,O^{2'}$ - di - phenylcarbamoyl - c.AMP for example, when infused at 10 mg./kg. over 20 minutes gave a 90—100% decrease in the resistance of a blood sample to flowing through a microscope filter compared with a control sample. Under the same conditions, this compound showed no effect on the concentration of glucose in the blood. This result is in marked contrast to the well-known $N^6,O^{2'}$ - di - n - butyryl - c.AMP which under the same conditions produced a substantial increase in the concentration of glucose in the blood without having any effect upon the aggregation of the platelets. The *in vivo* inhibition of platelet aggregation is useful in the prevention of thrombus formation.

The compounds of the invention do not produce overt toxic effects at the doses required to show activity, and $N^6,O^{2'}$ - di - phenylcarbamoyl - c.AMP has been administered to rabbits by intravenous injection at doses up to 100 mg./kg. without any toxic symptoms being observed.

When a compound of the invention is used to inhibit the aggregation of blood platelets *in vitro*, it is conveniently added to the blood so that its concentration in the blood is from 10^{-2} to 10^{-5} molar. When used to inhibit the aggregation of blood platelets in warm blooded animals, it is conveniently administered intravenously, by infusion or injection, at a dose from 2 mg./kg. to 500 mg./kg. or, for the more potent compounds, at a dose from 2 mg./kg. to 50 mg./kg. In man this is equivalent to a dose of from 1 mg./kg. to 20 mg./kg. of the preferred compounds which is conveniently repeated every 12 hours.

When used *in vivo*, a compound of the invention is conveniently administered in the form of a pharmaceutical composition comprising a cyclic AMP derivative of the invention in a sterile injectable medium.

A preferred composition is a sterile injectable solution of the cyclic AMP derivative. Such a preferred composition may contain from 7 mg./ml. to 140 mg./ml. of cyclic AMP derivative.

The invention is illustrated but not limited by the following Examples in which the N.m.r. spectra were measured using solutions in fully deuterated dimethyl sulphoxide with tetramethylsilane as internal reference, and the eluate from the chromatography columns was monitored by measuring the U.V. absorption at 260 nm.

Example 1

c.AMP (1 g.) was dissolved in water (5 ml.) and di - isopropylethylamine (5 ml.) added. The mixture was evaporated to a gum and dried *in vacuo* (12 mm. Hg; 40°C.) for 24 hours. The gum was dissolved in dry N,N - dimethylformamide (25 ml.) containing di - isopropylethylamine (0.12 ml), and the clear solution was treated with phenyl isocyanate (1.5 ml.). The reaction mixture was kept at room temperature for 72 hours. The solvent was removed under reduced pressure and the residue dissolved in acetonitrile: water (22: 3 v: v). The solution was applied to a silicic acid column (135×2.5 cm.) which was eluted with the same solvent. The eluate fractions (500—750 ml.) and (1—2 l.) were set aside and the fraction (750 ml.—1,000 ml.) was evaporated. The residue thus obtained was dissolved by warming in acetonitrile, and the solution was set aside at 4°C. After 16 hours the precipitate which formed was collected by filtration and dried to give $N^6,O^{2'}$ - di - phenylcarbamoyl - c.AMP di - isopropylethylammonium salt as an off-white powder (500 mg; 31%). Found C, 51.0; H, 6.2; N, 15.4%. $C_{24}H_{22}N_7O_8P \cdot C_8H_{19}N \cdot 3H_2O$ requires C, 51.1; H, 6.3; N, 15.0%; U.V. λ_{max} (ethanol) 278 n.m. (ε 26,900); N.m.r. δ 6.28 (s. H-1') and 5.74 (H-2') p.p.m.

Example 2

The eluate fractions (500—750 ml.) and (1—2 l.), set aside in Example 1, were combined, evaporated to dryness, and the residue consisting of di- and tri-phenylcarbamoyl-c.AMP was dissolved in 0.1M sodium hydroxide solution. After 1 hour at room temperature, the resulting solution was neutralised by the addition of sulphonated polystyrene resin (H^+ form), which was stirred with the solution, removed by filtration and washed with water. The combined filtrate and washings were evaporated to a small volume (4 ml.) and applied to a column of diethylaminoethyl - cellulose (HCO_3^- form) (32×4 cm.) which was eluted with a linear gradient of triethylammonium bicarbonate (0→1 Molar, in 5 l.). The major product emerged between 2.84 and 3.7 l. eluate, and this fraction was evaporated to dryness and the residual solid de-salted by repeated dissolution and evaporation from ethanol. The final residue was precipitated from ethanol to give N^6 - mono - phenylcarbamoyl - c.AMP triethylammonium salt, as an off-white powder (400 mg; 24%). Found C, 49.1; H, 6.2; N, 17.0%. $C_{17}H_{17}N_6O_7P \cdot C_6H_{15}N \cdot H_2O$ requires C, 48.7; H, 6.0; N, 17.3% U.V. λ_{max} (ethanol) 278 n.m. (ε 23,500); N.m.r. δ 6.40 (s. H-1') p.p.m.

Example 3

c.AMP (1 g.) was dissolved in a mixture of water (5 ml.) and di - isopropylethylamine (5 ml.). The solution was evaporated to a gum which was dried *in vacuo* (12 mm. Hg; 40°C.) overnight. The gum was dissolved in dry pyridine (50 ml.) and isobutyl chloroformate (0.84 ml.) added. After 48 hours at room temperature, a further quantity of the acid chloride (0.84 ml.) was added and the mixture set aside for a further 48 hours at room temperature. A crystalline precipitate was filtered off and discarded, and the filtrate evaporated to dryness. The residue was dissolved in water (20 ml.), and traces of pyridine were removed by extraction of the aqueous solution with ether (2×100 ml.). The water layer was then extracted with chloroform (5×50 ml.). The first three chloroform extracts were combined, evaporated to dryness and the residue, dissolved in chloroform, was applied to a column of silicic acid (20×2 cm.) with chloroform: ethanol (4: 1 v/v) as eluant. A major product emerged between 200—500 ml. eluate and this was obtained as a white powder after filtration of the precipitate obtained by the addition of petrol ether (b.p. 60—80°C.) to the eluate. The final two chloroform extracts were found to contain the same product as isolated from the column and so were combined directly with the precipitated material to give, after reprecipitation from petrol, $N^6,O^{2'}$ - di - isobutoxycarbonyl - c.AMP as its di - isopropylethylammonium salt (470 mg. 26%). Found C, 51.5; H, 7.5; N, 12.8%. $C_{20}H_{28}N_5O_{10}P \cdot C_8H_{19}N$ requires C, 51.0; H, 7.5; N, 12.8%; U.V. λ_{max} (ethanol) 267 n.m. (ε 17,400); N.m.r. δ 6.34 (s. H-1') and 5.68 (H-2') p.p.m.

Example 4

c.AMP (1 g.) was dissolved in a mixture of water (5 ml.) and di - isopropylethylamine (5 ml.). The solution was evaporated to a gum which was dried *in vacuo* (12 mm. Hg; 40°C.) overnight. The gum was dissolved in dry pyridine (50 ml.) and n-propyl chloroformate (1 ml.) added. Two further aliquots (1 ml.) of the chloroformate were added at 24 hour intervals. After a total of 72 hours at room temperature the mixture was evaporated to a gum which was dissolved in water (20 ml.). The solution was extracted with ether (2×50 ml.), the ether extracts being discarded, and then extracted with chloroform (6×30 ml.). The first extract was set aside and the remaining five chloroform extracts were combined and evaporated to dryness. The residue was triturated with petrol ether (b.p. 60—80°C.). The solid so produced was collected by filtration and air-dried to give $N^6,O^{2'}$ - di - n - propoxycarbonyl - c.AMP (600 mg.) as the di - isopropylethylammonium salt. A portion (450 mg.) of the product was dissolved in water and passed through a column of sulphonated polystyrene resin (H^+ form), and the eluate freeze-dried to give $N^6,O^{2'}$ - di - n - propoxycarbonyl - c.AMP (370 mg.). Found

C, 42.4; H, 5.0; N, 14.1; P, 6.5%. $C_{18}H_{24}N_5O_{10}P$ requires C, 43.1; H, 4.85; N, 14.0; P, 6.0% U.V. λ_{max} (ethanol) 267 n.m. (ε 17,450); N.m.r. δ 6.3 (s. H-1') and 5.72 (H-2') p.p.m.

Example 5

The first extract set aside in Example 4 was evaporated to a gum containing as the major product $N^6O^{2'}$ - di - n - propoxycarbonyl - c.AMP, together with a second product tentatively identified as $N^6,N^6,O^{2'}$ - tri - n - propoxycarbonyl - c.AMP. The gum was dissolved in ethanol (9 ml.) and a solution of 1M sodium hydroxide (1 ml.) added. After 15 minutes the reaction solution was neutralised by the addition of sulphonated polystyrene resin (H^+ form), which was stirred with the solution, removed by filtration and washed with water. The combined filtrate and washings were freeze-dried to give N^6 - mono - n - propoxycarbonyl - c.AMP (230 mg.). Found C, 36.3; H, 4.3; N, 15.1% for the sodium salt, $C_{14}H_{17}N_5O_8P$ Na. 1.5 H_2O requires C, 36.2; H, 4.3; N, 15.1%; U.V. λ_{max} (ethanol) 267 n.m. (ε 17,750); N.m.r. δ 6.06 (s. H-1') p.p.m.

Example 6

n-Butyl isocyanate (1 ml.) was added to a solution of c.AMP di - isopropylethylammonium salt (1.2 g.) in N,N - dimethylformamide (40 ml.), and the mixture heated at 100°C. for 12 hours. A further quantity of the isocyanate (2 ml.) was then added and the solution heated at 100°C. for a further 3 days. The solvent was then evaporated under reduced pressure, and the residual oil was dissolved in water (30 ml.). The solution was extracted with chloroform (6×50 ml.) and the extracts evaporated to dryness to give $N^6,O^{2'}$ - di - n - butylcarbamoyl - c.AMP (450 mg) as its di - isopropylethylammonium salt. A portion of this salt (380 mg.) was passed through a column of sulphonated polystyrene resin (H^+ form), and the eluate and water washings were freeze-dried to give $N^6,O^{2'}$ - di - n - butylcarbamoyl - c.AMP as the free acid (300 mg.). Found C, 44.2; H, 5.8%. $C_{20}H_{30}N_7O_8P$. H_2O requires C, 44.0; H, 5.9%; U.V. λ_{max} (ethanol) 269 n.m. (ε 20,500); N.m.r. δ 6.12 (s. H-1') and 5.55 (H-2') p.p.m.

Example 7

Ethyl chloroformate (2.5 ml.) was added to a solution of c.AMP triethylammonium salt (1 g.) in dry pyridine (50 ml.) and the mixture left at room temperature for 72 hours. The solvent was then evaporated under reduced pressure, and the residue dissolved in water (30 ml.). The first two chloroform ex- (6×30 ml.). The first two chloroform extracts were set aside and the remaining four chloroform extracts were combined and evaporated to dryness. The residue was dissolved in water (50 ml.), passed through a column of sulphonated polystyrene resin (H^+ form) and the eluate freeze-dried to give $N^6,O^{2'}$ - di - ethoxycarbonyl - c.AMP (250 mg.) as the free acid. Found C, 37.5; H, 4.4; N, 13.9%. $C_{16}H_{20}N_5P$. $2H_2O$ requires C, 37.7; H, 4.6; N, 13.8%; U.V. λ_{max} (ethanol) 267 n.m. (ε 16,900); N.m.r. δ 6.32 (s, H-1') and 5.66 (H-2') p.p.m.

Example 8

2,2,2 - Trichloroethyl chloroformate (1.5 ml.) was added to a solution of c.AMP triethylammonium salt (1 g.) dissolved in dry pyridine (50 ml.). The mixture was left at room temperature for 48 hours, and then evaporated to dryness under reduced pressure. The residue was dissolved in chloroform and fractionated on a column of silicic acid (12×2 cm.) with chloroform: ethanol (19:1 v/v) as eluant. A major product was obtained after 300 ml. eluate and was isolated after evaporation, as a white powder (1 g.). A portion of this (400 mg.) was dissolved in aqueous ethanol and passed through a column of sulphonated polystyrene resin (H^+ form). The eluate was freeze-dried to give $N^6,O^{2'}$ - di - 2,2,2 - trichloroethoxycarbonyl - c.AMP (365 mg.) as the free acid. Found C, 28.6; H, 2.2; N, 10.3%. $C_{16}H_{14}N_5O_{10}PCl_6$ requires C, 28.2; H, 2.1; N, 10.3%; U.V. λ_{max} (ethanol) 267 n.m. (ε 17,700); N.m.r. δ 6.48 (s, H-1') and 5.81 (H-2') p.p.m.

Example 9

A second portion of the major product (400 mg.) obtained in Example 8 was dissolved in ethanol (9 ml.) and 1M sodium hydroxide (1 ml.), and the solution left at room temperature for 30 minutes. The mixture was neutralised by passage through a column of sulphonated polystyrene resin (H^+ form), and the eluate and washings, partially evaporated and then freeze-dried to give N^6 - mono - 2,2,2 - trichloroethoxycarbonyl - c.AMP (265 mg.) as the free acid. Found C, 30.4; H, 3.1; N, 13.2%. $C_{13}H_{13}N_5O_8PCl_3$. H_2O requires C, 29.9; H, 2.9; N, 13.4% U.V. λ_{max} (ethanol) 267 n.m. (ε 17,100); N.m.r. δ 6.03 (s, H-1') p.p.m.

Example 10

n-Butyl chloroformate (5 ml.) was added to a solution of C.AMP triethylammonium salt (1 g.) in dry pyridine (50 ml.), and the mixture left at room temperature for 48 hours. The solvent was removed under reduced pressure, and the residue dissolved in water (30 ml.) and extracted with chloroform (6×30 ml.). The combined chloroform extracts were evaporated in the presence of silicic acid (10 g.) to a dry powder which was applied to a column of silicic acid (15×1.5 cm.) and fractionated with acetonitrile: water (88:12). The first major eluate fraction (200—500 ml.)

was set aside and the second major fraction (500—800 ml.) was evaporated to dryness. The residue was dissolved in water, and the solution passed through a column of sulphonated polystyrene resin (Na^+ form). The eluate was then freeze-dried to give $N^6,O^{2'}$ - di - n - butoxycarbonyl - c.AMP (280 mg.) as the sodium salt. Found C, 41.8; H, 4.9; N, 12.6%. $C_{20}H_{27}N_5O_{10}P$, $Na . H_2O$ requires C, 42.2; H, 5.1; N, 12.3%. U.V. $\lambda_{max.}$ (ethanol) 267 n.m. (ε 17,900); N.m.r. δ 6.28 (s, H-1') and 5.62 (H-2') p.p.m.

Example 11

The first major eluate fraction (200—500 ml.) set aside in Example 10 contained both di- and tri-n-butoxycarbonyl-c.AMP. It was evaporated and the residue was treated with aqueous ethanolic 0.1 M sodium hydroxide (10 ml.) at room temperature for 4 hours. The mixture was neutralised with a sulphonated polystyrene resin (H^+ form). The aqueous eluate was freeze-dried to give N^6 - mono - n - butoxycarbonyl - c.AMP (220 mg.). Found C, 39.9; H, 4.4; N, 15.6%. $C_{15}H_{20}N_5O_8P . H_2O$ requires C, 40.3; H, 4.9; N, 15.6%; U.V. $\lambda_{max.}$ (ethanol) 267 n.m. (ε 17,450); N.m.r. δ 6.00 (s, H-1') p.p.m.

Example 12

n-Heptyl chloroformate (5 ml.) was added to a stirred solution of c.AMP triethylammonium salt (1 g.) in dry pyridine (50 ml.). After 48 hours the solution was treated with water (5 ml.) and the mixture evaporated to dryness. The residue was dissolved in acetonitrile and fractionated on a column of silicic acid (30×2 cm.) with acetonitrile: water (88 : 12) as eluant. The major fraction obtained after 300 ml. eluate was set aside, and a second fraction obtained after 500 ml. eluate was further fractionated by preparative t.l.c. on silicic acid (GF_{254} plates, 20×20 cm., acetonitrile: water, 88 : 12) into two fraction, A and B. Fraction A (R_F 0.66) was isolated by elution with ethanol. The solid obtained after evaporation under reduced pressure was dissolved in water, and the solution was passed through a column of sulphonated polystyrene resin (H^+ form). The eluate was freeze-dried to give $N^6,O^{2'}$ - di - n - heptyloxycarbonyl - c.AMP (120 mg.) as the free acid. Found C, 49.9; H, 6.0; N, 11.6%. $C_{26}H_{40}N_5O_{10}P$ requires C, 50.8; H, 6.5; N, 11.4%. U.V. $\lambda_{max.}$ (ethanol) 267 n.m. (ε 17,400); N.m.r. δ 6.32 (s, H-1') and 5.62 (H-2') p.p.m.

Example 13

The major fraction obtained after 300 ml. eluate and set aside in Example 12 was evaporated. The residue of di- and tri-n-heptyloxycarbonyl-c.AMP was hydrolysed with aqueous ethanolic 0.1 M sodium hydroxide (10 ml.) at room temperature for 30 minutes. The hydrolysate was neutralised with sulphonated polystyrene resin (H^+ form), and the eluate freeze-dried to give N^6 - mono - heptyloxycarbonyl - c.AMP (180 mg.). Found C, 42.7; H, 5.3; N, 13.5%, $C_{18}H_{26}N_5O_8P . 2H_2O$ requires C, 42.6; H, 5.9; N, 13.7%; U.V. $\lambda_{max.}$ (ethanol) 267 n.m. (ε 17,950); N.m.r. δ 6.03 (s, H-1') p.p.m.

Example 14

Ethyl isocyanate (2 ml.) was added to a solution of c.AMP triethylammonium salt (1 g.) in dry pyridine (50 ml.). The mixture was heated under reflux for 16 hours, and left to cool. The pyridine was evaporated under reduced pressure, and the gummy residue was fractionated on a column of silicic acid (15×1.5 cm.) with acetonitrile: water (88 : 12 v/v) as eluant. A major fraction was isolated after evaporation of the eluate, dissolution of the residue in water, passage of the solution through a column of sulphonated polystyrene resin (Na^+ form) and freeze-drying of the eluate to give $N^6,O^{2'}$ - diethylcarbamoyl - c.AMP (610 mg.). Found C, 35.9; H, 4.4; N, 18.1%, $C_{16}H_{21}N_7O_8PNa . 2H_2O$ requires C, 36.3; H, 4.7; N, 18.5%. U.V. $\lambda_{max.}$ 268 n.m. (ε 21,000); N.m.r. δ 6.13 (s, H-1') and 5.54 (H-2') p.p.m.

Example 15

A portion (200 mg.) of the product obtained in Example 14 was dissolved in 0.1 M potassium hydroxide and left at room temperature for 48 hours. The solution was neutralised by passage through a column of sulphonated polystyrene resin (H^+ form), and the eluate and washings were freeze-dried to give N^6 - mono - ethylcarbamoyl - c.AMP (120 mg.) as the free acid. Found C, 33.9; H, 4.8; N, 19.0%, $C_{13}H_{17}N_6O_7P . 3H_2O$ requires C, 34.2; H, 5.1; N, 18.5%. U.V. $\lambda_{max.}$ (ethanol) 268.5 n.m. (ε 19,500). N.m.r. δ 6.0 (s, H-1') p.p.m.

Example 16

p - Chlorophenylisocyanate (2 g.) was added to a solution of c.AMP triethylammonium salt (1 g.) in dry pyridine (50 ml.), and the mixture heated at 80°C. for 30 minutes. Water (5 ml.) was added, and the solvent evaporated. The residue was suspended in chloroform and a precipitate of 1,2 - bis - *p* - chlorophenylurea was removed by filtration. The chloroform filtrate and washings were evaporated to dryness, and the residue fractionated on a silicic acid column (10×1.5 cm.) with acetonitrile as eluant. After 1.5 l. of eluate, all of the urea had been removed and the eluate was changed to acetonitrile: water (22 : 3 v/v). The eluate fraction (300—1,000 ml.) was evaporated to give $N^6,O^{2'}$ - di - *p* - chlorophenylcarbamoyl - c.AMP as a crystalline solid (250 mg.) and as an amorphous solid (1.65 g.). Found C, 42.3; H, 3.3; N, 13.8%.

$C_{24}H_{20}N_7O_8PCl_2 . 3H_2O$ requires C, 41.7; H, 3.7; N, 14.2%. U.V. $\lambda_{max.}$ (ethanol) 279 n.m. (ε 29,250); N.m.r. δ 6.34 (s, II-1') and 5.74 (H-2') p.p.m.

Example 17

$N^6,O^{2'}$ - Di - *p* - chlorophenylcarbamoyl - c.AMP (800 mg.) was dissolved in ethanol (9 ml.) and 1 M sodium hydroxide (1 ml.) and the solution left at room temperature for 24 hours. The solution was rendered neutral by passage through a column of sulphonated polystyrene resin (H^+ form), and the eluate was freeze dried to give N^6 - mono - *p* - chlorophenylcarbamoyl - c.AMP as the free acid form (240 mg.). Found C, 39.1; H, 3.7; N, 15.9%. $C_{17}H_{16}N_6O_7PCl . 2H_2O$ requires C, 39.4%; H, 3.8; N, 16.2%. U.V. $\lambda_{max.}$ (ethanol) 278 n.m. (ε 23,500); N.m.r. δ 6.09 (s, H-1') p.p.m.

Example 18

1 - Naphthylisocyanate (2 ml.) was added to a solution of c.AMP triethylammonium salt (1 g.) dissolved in dry pyridine (50 ml.). The mixture was left at room temperature for 48 hours, and the precipitate of 1,2 - bis - naphthylurea filtered off. The pyridine was removed under reduced pressure, and the residue fractionated on a silicic acid column (30 × 2 cm.) with acetonitrile: water (22: 3) as eluant. The eluate was evaporated to give an amorphous powder (900 mg.). Part of this material (600 mg.) was dissolved in aqueous ethanol and passed through a column of sulphonated polystyrene resin (Na^+ form). The eluate was partially evaporated and then freeze-dried to give $N^6,O^{2'}$ - di - naph - 1 - ylcarbamoyl - c.AMP (500 mg.) as the sodium salt. Found C, 50.8; H, 3.6; N, 13.1%. $C_{32}H_{25}N_7O_8PNa . 3H_2O$ requires C, 51.7; H, 4.2; N, 13.2%. U.V. $\lambda_{max.}$ (ethanol) 275 n.m. (broad) (ε 18,500). N.m.r. δ 6.37 (s, H-1') and 5.80 (H-2') p.p.m.

Example 19

Phenylisocyanate (4 ml.) was added to a solution of c.AMP triethylammonium salt (1.2 g.) in dry pyridine (60 ml.), and the mixture left at room temperature for 48 hours. Water (5 ml.) was added, and the mixture evaporated to dryness. The solid was extracted continuously with ether in a Soxhlet apparatus for 48 hours after which time all of the 1,2 - bisphenylurea had been removed. The residue in the thimble was dissolved in aqueous acetonitrile and the undissolved solid collected by centrifugation. The viscous supernatant liquid was diluted further with aqueous acetonitrile and the solution passed through a column of sulphonated polystyrene (H^+ form). The eluate and water washings were partially evaporated under reduced pressure and then freeze-dried to give $N^6,O^{2'}$ - diphenylcarbamoyl - c.AMP (1.8 g.).

Example 20

c.AMP (250 mg.) was dissolved in a mixture of water (1 ml.) and triethylamine (1 ml.). The mixture was evaporated to a gum, which was dried *in vacuo* over concentrated sulphuric acid at room temperature to give the triethylammonium salt. This salt was dissolved in dry pyridine (10 ml.) containing octyl isocyanate (1 ml.) and the mixture was heated under reflux for 6 hours. The solution was diluted with water (140 ml.), and the mixture allowed to stand at room temperature for 17 hours. The solid material was filtered off, and the filtrate evaporated under reduced pressure. The residue was dissolved in chloroform and fractionated on a column of silicic acid (30 g.) with chloroform: methanol (4: 1 v/v) as eluant. Fractions containing only the major product were evaporated to dryness. The residue was dissolved in chloroform, and the solution passed through a column of sulphonated polystyrene resin (Na^+ form). The eluate was evaporated, the residue re-dissolved in water, and the solution freeze-dried to give $N^6,O^{2'}$ - di - n - octylcarbamoyl - c.AMP as the sodium salt (164 mg.). Found C, 50.2; H, 7.2; N, 14.4%. $C_{28}H_{45}H_7O_8PNa . \frac{1}{2}H_2O$ requires C, 50.1, H, 6.9; N, 14.6%. N.m.r. 6.16 (s, H-1') p.p.m.

Example 21

$N^6,O^{2'}$ - Di - n - octylcarbamoyl - c.AMP (100 mg.) was dissolved in dioxan (1 ml.) and 1N sodium hydroxide (1 ml.), and the solution left at room temperature for 48 hours. The solution was rendered neutral by passage through a column of sulphonated polystyrene resin (H^+ form), and the eluate was freeze-dried to give N^6 - mono - n - octylcarbamoyl - c.AMP as the free acid (65 mg.). Found C, 45.4; H, 6.3; N, 15.7%. $C_{19}H_{29}N_6O_7P . H_2O$ requires C, 45.5; H, 6.2; N, 16.7%. U.V. $\lambda_{max.}$ (water) 269 n.m. (ε 20,900) inflexion 276 n.m.; N.m.r. δ 6.06 (s, H-1') p.p.m.

Example 22

c.AMP triethylammonium salt was prepared as described in Example 20 from the free acid (500 mg.). It was dissolved in dry pyridine (20 ml.) and *o* - chlorophenylisocyanate (2 ml.). The solution was left at room temperature for 48 hours, then diluted with water and left at 4°C. for a further 24 hours. The resulting precipitate was filtered off, and the filtrate evaporated to a gel, which was re-dissolved in acetonitrile and the solution passed down a column of sulphonated polystyrene resin (Na^+ form). The eluate was concentrated to a gel and freeze-dried to give $N^6,O^{2'}$ - di - *o* - chlorophenylcarbamoyl - c.AMP as the sodium salt (1.12 g.). Found C, 43.8; H, 3.3; N, 14.2%. $C_{24}H_{19}N_7O_8PCl_2Na$ requires C, 43.7; H, 2.9; N, 14.9%. U.V. $\lambda_{max.}$ (water) 277 n.m. (ε 23,600); N.m.r. δ 6.32 (s, H-1') p.p.m.

Example 23

The process described in Example 22 was repeated except that *m* - chlorophenylisocyanate (2 ml.) was used in place of *o* - chlorophenylisocyanate. After a total of 48 hours at room temperature, the mixture was diluted with water and stored at 4°C. for 24 hours. The resulting precipitate was filtered off, and the filtrate evaporated to give 1.06 g. of solid. A portion of this solid (500 mg.) was dissolved in acetonitrile and the solution applied to a column of silicic acid (100 g.). The column was eluted with acetonitrile: water (80: 20), and the fractions containing the major product were passed down a column of sulphonated polystyrene resin (Na^+ form). The eluate was partially evaporated, and then freeze dried to give $N^6,O^{2'}$ - di - *m* - chlorophenylcarbamoyl - c.AMP as the sodium salt (350 mg.). Found C, 41.0; H, 3.9; N, 13.1%. $C_{24}H_{19}N_7O_8PCl_2Na \cdot 2\frac{1}{2}H_2O$ requires C, 41.0; H, 3.4; N, 13.3%. U.V. $\lambda_{max.}$ (water) 277 n.m. (ε 30,100); N.m.r. δ 6.31 (s, H-1') p.p.m.

Example 24

The process described in Example 22 was repeated except that *p* - methoxyphenylisocyanate (2 ml.) was used in place of *o* - chlorophenylisocyanate, and the initial product was converted into the sodium salt using a mixture of acetonitrile and methanol in place of acetonitrile. The eluate was evaporated to a solid (1.15 g.). A portion of this solid (0.51 g.) was dissolved in methanol and the resulting solution was evaporated in the presence of silicic acid (5 g.) to a dry powder. This powder was applied to a column of silicic acid (25 g.) and fractionated with acetonitrile: water (9 : 1) as eluant. Fractions containing the major product were filtered to remove white crystals (100 mg.) and evaporated to a white solid. The solid was dissolved in aqueous methanol, and the solution passed down a column of sulphonated polystyrene resin (Na^+ form). The eluate was partially evaporated and freeze-dried to give $N^6,O^{2'}$ - di - *p* - methoxyphenylcarbamoyl - c.AMP as the sodium salt (285 mg.). Found C, 46.0; H, 4.3; N, 14.1%. $C_{26}H_{25}N_7O_{10}PNa \cdot 2H_2O$ requires C, 45.5; H, 4.2; N, 14.3%. U.V. $\lambda_{max.}$ (water) 277 n.m. (ε 28,700); N.m.r. δ 6.29 (s, H-1') p.p.m.

Example 25

Freshly distilled allylisocyanate (3 ml.) was added to a solution of c.AMP triethylammonium salt (1.3 g.) in dry pyridine (50 ml.) and the mixture was heated under reflux for $3\frac{1}{2}$ hours. The pyridine was distilled off under reduced pressure, and the residue was fractionated on a column of silicic acid (20×3 cm.) with acetonitrile: water (88:12 v/v) as eluant. The substance contained in the eluate between 170 ml. and 380 ml. was recovered by evaporation under reduced pressure, re-dissolved in water (20 ml.) and passed down a column of sulphonated polystyrene resin (Na^+ form—bed volume 15 ml.). Freeze-drying of the eluate and washings gave $N^6,O^{2'}$ - di - allylcarbamoyl - c.AMP as the sodium salt (0.88 g.). Found C, 38.5; H, 4.3; N, 17.4%. $C_{18}H_{21}N_7O_8PNa$, $2H_2O$ requires C, 39.0; H, 4.6; N, 17.7%. U.V. $\lambda_{max.}$ (water) 269 n.m. (ε 22,100); N.m.r. δ 6.14 (s, H-1') p.p.m.

Example 26

Freshly distilled benzylisocyanate (3.5 ml.) was added to a solution of c.AMP triethylammonium salt (1.3 g.) in dry pyridine (50 ml.), and the mixture was heated under reflux for $2\frac{1}{2}$ hours. Removal of the pyridine under reduced pressure left an oil which yielded a solid upon trituration with ethyl acetate (25 ml.). The solid was collected, washed with ethyl acetate, and purified on a column of silicic acid (20×3 cm.) with acetonitrile: water (88 : 12 v/v) as eluant. Material contained in the eluate between 150 ml. and 550 ml. was recovered by evaporation under reduced pressure, and dissolved as far as possible in water (40 ml.). The solution was clarified by filtration and passed down a column of sulphonated polystyrene resin (N^+ form—bed volume 25 ml.). The eluate and washings were freeze-dried to give $N^6,O^{2'}$ - di - benzylcarbamoyl - c.AMP as the sodium salt (0.70 g.). Found C, 47.3; H, 4.7; N, 14.8%. $C_{26}H_{25}N_7O_8PNa$, $2H_2O$ requires C, 47.8; H, 4.5; N, 15.0%. U.V. $\lambda_{max.}$ (water) 269 n.m. (ε 23,500); N.m.r. δ 6.16 (s, H-1') p.p.m.

Example 27

Distilled 2 - chloroethylisocyanate (3.5 ml.) was added to a solution of c.AMP triethylammonium salt (1.3 g.) in dry pyridine (50 ml.). After 46 hours at ambient temperature, the solution was evaporated below 30°C. under reduced pressure. The residue was purified on a column of silicic acid (20×3 cm.) with acetonitrile: water (88:12 v/v) as eluant. The substance contained in the eluate between 200 ml. and 310 ml. was recovered by evaporation under reduced pressure, re-dissolved in water (16 ml.), and passed down a column of sulphonated polystyrene (H^+ form—bed volume 20 ml.). The total eluate was first concentrated under reduced pressure below 20°C. and then freeze-dried to give $N^6,O^{2'}$ - di - 2 - chloroethylcarbamoyl - c.AMP as the free acid (1.07 g.). Found C, 34.6; H, 3.8; N, 17.6; Cl, 13.4%. $C_{16}H_{20}N_7O_8Cl_2P \cdot O \cdot 5H_2O$ requires C, 35.0; H, 3.8; N, 17.9; Cl, 12.9%. U.V. $\lambda_{max.}$ (water) 268 n.m. (ε 23,100); N.m.r. δ 6.34 (s, H-1') p.p.m.

Example 28

Distilled allyl chloroformate (5 ml.) was added dropwise to a solution of c.AMP tri-

ethylammonium salt (1.3 g.) in dry pyridine (50 ml.) which was stirred in ice-water. The mixture was stirred at ambient temperature for 42 hours during which time the red solution and gummy solid were replaced by a yellow solution in which crystalline pyridine hydrochloride was suspended. The whole was evaporated under reduced pressure and the residue was re-dissolved in water (25 ml.). The solution was washed with ether (ca. 20 ml.×2), and repeated evaporation of the aqueous layer under reduced pressure then gave a gum which was fractionated on a column of silicic acid (20×3 cm.) with acetonitrile: water (88:12 v/v) as eluant. The fraction from 130 ml. to 160 ml. of eluate was evaporated under reduced pressure, and the residue re-dissolved in water (10 ml.). The solution was washed with ether (ca. 10 ml.), and then extracted with chloroform (7 ml.×12). The first extract showed two spots on t.l.c., but the others showed only one spot and so they were combined, dried over sodium sulphate, and evaporated to give a gum which solidified on trituration with ether. The solid was re-dissolved in water (5 ml.) and the solution passed down a column of sulphonated polystyrene resin (Na^+ form—bed volume 5 ml.). The total eluate was freeze-dried to give N^6,$O^{2'}$ - di - allyloxycarbonyl - c.AMP as the sodium salt (0.13 g.). Found C, 38.2; H, 3.7; N, 12.2%. $C_{18}H_{19}N_5O_{10}PNa \cdot 2H_2O$ requires C, 38.8; H, 4.2; N, 12.6%. U.V. $\lambda_{max.}$ (water) 267 nm. (ε 18,100); N.m.r. δ 6.33 (s, H-1') p.p.m.

Example 29

N^6 - Mono - phenylcarbamoyl - c.AMP (670 mg.) was dissolved in a mixture of water (2 ml.) and triethylamine (2 ml.). The solution was evaporated to a gum which was dried *in vacuo* over concentrated sulphuric acid at room temperature to give the triethylammonium salt, This salt was dissolved in a mixture of dry pyridine (50 ml.) and n-butyric anhydride (0.26 ml.). Further quantities of n-butyric anhydride were added after 24 hours (0.5 ml.) and 48 hours (1.25 ml.). After a total of 72 hours, the mixture was treated with water (5 ml.) and after a further 2 hours the mixture was evaporated to dryness. The residue was dissolved in water (100 ml.) and extracted with chloroform (3×100 ml.). The combined chloroform extracts were concentrated and fractionated on a column of silicic acid (35 g.) with acetonitrile as eluant. The eluant was changed to acetonitrile: water (4:1) and a major fraction (100—150 ml.) was collected. A white precipitate in this fraction was collected by filtration to give $O^{2'}$ - n - butyryl - N^6 - phenylcarbamoyl - c.AMP as the free acid (110 mg.). The filtrate was evaporated to dryness, the residue re-dissolved in a mixture of ethanol and water, and the solution passed through a column of sulphonated polystyrene resin (Na^+ form). The eluate was partially evaporated then freeze-dried to give $O^{2'}$ - n - butyryl - N^6 - phenylcarbamoyl - c.AMP as the sodium salt (357 mg.). Found C, 45.1; H, 4.5; N, 14.6%. $C_{21}H_{22}N_6O_8PNa \cdot H_2O$ requires C, 45.1; H, 4.3; N, 15.1%. U.V. $\lambda_{max.}$ (water) 277 n.m. (ε 26,500); N.m.r. δ 6.28 (s, H-1') p.p.m.

Example 30

N^6 - Mono - phenylcarbamoyl - c.AMP (450 mg.) was converted into its anhydrous triethylammonium salt as described in Example 29. The salt was dissolved in dry pyridine (10 ml.) and reacted with n-butyl chloroformate (2 ml.) at room temperature for 24 hours. The reaction mixture was diluted with water and evaporated to a gum which was re-dissolved in water (50 ml.). The solution was extracted with chloroform (3×50 ml.) and the extracts evaporated to dryness. The residue was re-dissolved in acetonitrile and fractionated on a column of silicic acid (40 g.) using acetonitrile (200 ml.) as the first eluant, followed by acetonitrile: water (88:12). The major product appeared in the acetonitrile: water fractions, and these were passed down a column of sulphonated polystyrene resin (Na^+ form). The eluate was partially evaporated and freeze-dried to give $O^{2'}$ - n - butyloxycarbonyl - N^6 - phenylcarbamoyl - c.AMP as the sodium salt (226 mg.). Found C, 44.5; H, 4.0; N, 14.1%. $C_{22}H_{24}N_6O_9PNa \cdot H_2O$ requires C, 44.9; H, 4.4; N, 14.3%. U.V. $\lambda_{max.}$ (water) 277 n.m. (ε 26,000); N.m.r. δ 6.35 (s, H-1') p.p.m.

Example 31

N^6 - Mono - phenylcarbamoyl - c.AMP (230 mg.) was converted into the triethylammonium salt as described in Example 29. The salt was dissolved in dry pyridine (10 ml.) and reacted with n-hexyl chloroformate (2.5 ml.) and the product isolated as described for Example 30 to give $O^{2'}$ - n - hexyloxycarbonyl - N^6 - phenylcarbamoyl - c.AMP as the sodium salt (214 mg.). Found C, 44.0; H, 5.1; N, 12.0%. $C_{24}H_{28}N_6O_9PNa \cdot 3H_2O$ requires C, 44.2; H, 5.2; N, 12.9%. U.V. $\lambda_{max.}$ (water) 277 n.m. (ε 24,800); N.m.r. δ 6.33 (s, H-1') p.p.m.

Example 32

N^6 - Mono - phenylcarbamoyl - c.AMP (450 mg.) was converted into the triethylammonium salt as described in Example 29. The salt was dissolved in dry pyridine (10 ml.) and reacted with n - octylisocyanate (3 ml.) at room temperature for 17 days. Water (100 ml.) was added, and the mixture left at 4°C. for 24 hours. The solids were filtered off, and the filtrate evaporated to dryness. The residue was dissolved in acetonitrile and fractionated on a column of silicic

acid (40 g.) using acetonitrile (200 ml.) then acetonitrile: water (88:12) as eluants. The major product appeared in the fraction (125 ml.—350 ml.) of the acetonitrile: water eluate, and this fraction was passed through a column of sulphonated polystyrene resin (Na^+ form). The eluate was partially evaporated and freeze-dried to give $O^{2'}$ - n - octylcarbamoyl - N^6 - phenylcarbamoyl - c.AMP as the sodium salt (410 mg.). Found C, 47.3; H, 5.8; N, 15.1%. $C_{26}H_{32}N_7O_8PNa.2H_2O$ requires C, 47.2; H, 5.6; N, 14.8%. U.V. $\lambda_{max.}$ (water) 277 n.m. (ε 28,200); N.m.r. δ 6.16 (s, H-1') p.p.m.

Example 33

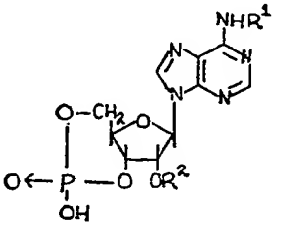
N^6 - Mono - *p* - chlorophenylcarbamoyl - c.AMP triethylammonium salt (0.88 g.) was dissolved in a mixture of dry pyridine (20 ml.) and phenyl isocyanate (2 ml.). The mixture was allowed to stand at room temperature for 72 hours, after which it was diluted with water and kept at 4°C. for 24 hours. The resulting precipitate was filtered off, and the filtrate evaporated to a solid which was dissolved in acetonitrile and fractionated on a column of silicic acid (200 g.) using acetonitrile then acetonitrile: water (9:1) as eluants. The major product appeared in the acetonitrile: water eluate, and was isolated as a solid after evaporation. The solid was dissolved in acetonitrile and the solution passed down a column of sulphonated polystyrene resin (Na^+ form). The eluate was partially evaporated and freeze-dried to give N^6 - *p* - chlorophenylcarbamoyl - $O^{2'}$ - phenylcarbamoyl - c.AMP as the sodium salt (600 mg.). Found C, 43.4; H, 3.5; N, 14.5%. $C_{24}H_{20}N_7O_8PCl.Na.2H_2O$ requires C, 43.7; H, 3.6; N, 14.9%. U.V. $\lambda_{max.}$ (water) 277 n.m. (ε 30,800); N.m.r. δ 6.32 (s, H-1') p.p.m.

Example 34

N^6,$O^{2'}$ - Di - isobutoxycarbonyl - c.AMP (350 mg.) was dissolved in 0.1 M sodium hydroxide solution (20 ml.) and the solution kept at room temperature for 24 hours. The solution was then neutralised with sulphonated polystyrene resin (H^+ form), filtered, and the filtrate evaporated to dryness in the presence of silicic acid (10 g.). The residue was applied to a column of silicic acid (20×4 cm.) packed in acetonitrile, and the column eluted with acetonitrile-water (22:3). The fraction eluted between 400—500 ml. of eluate was evaporated to give N^6 - mono - isobutoxycarbonyl - c.AMP (250 mg.) which was converted into its sodium salt by interaction with a sulphonated polystyrene resin (Na^+ form). Found C, 37.9; H, 4.8; N, 14.8%. $C_{15}H_{19}N_5O_8PNa.H_2O$ requires C, 38.3; H, 4.5; N, 15.0%. U.V. $\lambda_{max.}$ (ethanol) 267 n.m. (ε 16,800); N.m.r. δ 6.33 (s, H-1') p.p.m.

WHAT WE CLAIM IS:—

1. A cyclic AMP derivative of the formula:—



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wherein R^1 is an acyl group of the formula —CO . OR^3 or —CO . NHR^4, wherein R^3 is an alkyl radical of 1—8 carbon atoms, a haloalkyl radical of 1—4 carbon atoms, or an alkenyl radical of up to 4 carbon atoms, and R^4 is an alkyl radical of 1—8 carbon atoms, a haloalkyl radical of 1—4 carbon atoms, an alkenyl radical of up to 4 carbon atoms, a phenylalkyl radical of 7—9 carbon atoms, a phenyl radical, a naphthyl radical, or a phenyl radical bearing as a substituent, a halogen atom or an alkyl or alkoxy radical of 1—3 carbon atoms; and R^2 is hydrogen or an acyl group of the formula —CO . OR^3, —CO . NHR^4 or —CO . R^5, wherein R^3 and R^4 are as defined above and R^5 is an alkyl radical of 1—4 carbon atoms, or a base addition salt thereof.

2. A compound as claimed in claim 1 wherein R^3 or R^4 is an alkyl radical of 3—6 carbon atoms.

3. A compound as claimed in claim 1 or 2 wherein R^3 or R^4 is a methyl, ethyl, n-propyl, n-butyl, isobutyl, n-hexyl, n-heptyl, n-octyl, 2,2,2 - trichloroethyl 2 - chloroethyl or allyl radical, or R^4 is a benzyl, phenyl, naphthyl, *o* - chlorophenyl, *m* - chlorophenyl, *p* - chlorophenyl or *p* - methoxyphenyl radical, and R^5 is an n-propyl radical.

4. A compound as claimed in claim 1, 2 or 3 wherein R^1 and R^2 are both acyl groups of the formula —CO . OR^3 or both acyl groups of the formula —CO . NHR^4.

5. A compound as claimed in claim 1, 2 or 3 wherein R^1 is an acyl group of the formula —CO . OR^3 or —CO . NHR^4, and R^2 is a different acyl group of the formula —CO . OR^3, —CO . NHR^4 or —CO . R^5.

6. The cyclic AMP derivative, N^6,$O^{2'}$ - di - phenylcarbamoyl - c.AMP or a base addition salt thereof.

7. The cyclic AMP derivative, N^6,$O^{2'}$ - di - isobutoxycarbonyl - c.AMP or N^6,$O^{2'}$ - di - n - butoxycarbonyl - c.AMP, or a base addition salt thereof.

8. A base addition salt as claimed in any of claims 1 to 7 which is a pharmaceutically-acceptable base addition salt.

9. A base addition salt as claimed in any of claims 1 to 7 which is a barium salt or a salt with an organic amine.

10. A process for the manufacture of a cyclic AMP derivative claimed in claim 1 which comprises

1) for a compound of formula I wherein R^1 is an acyl group of the formula —CO . OR^3 or —CO . NHR^4 and R^2 is hydrogen, or R^1 and R^2 are identical acyl groups of the formula —CO . OR^3 or —CO . NHR^4, reacting cyclic AMP, or a base addition salt thereof, with at least 1.5 molecular equivalents of an acylating agent derived structurally from an acid of the formula R^3O . CO . OH or R^4NH . CO . OH; wherein R^3 and R^4 are as stated in claim 1;

2) for a compound of formula I wherein R^1 is an acyl group of the formula —CO . NHR^4 and R^2 is hydrogen, or R^1 and R^2 are identical acyl groups of the formula —CO . NHR^4, reacting cyclic AMP, or a base addition salt thereof, with at least 1.5 molecular equivalents of an isocyanate of the formula R^4 . N=C=O, wherein R^4 is as stated in claim 1;

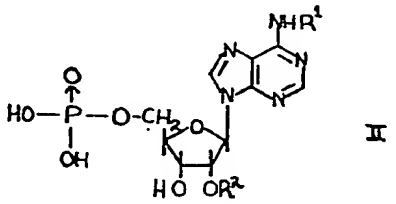
3) for a compound of formula I wherein R^2 is hydrogen, hydrolysing the corresponding cyclic AMP derivative of formula I, wherein R^2 is an acyl group, with dilute alkali, or hydrolysing a tri-acylated derivative of cyclic AMP with dilute alkali;

4) for a compound of formula I wherein R^2 is other than hydrogen:

a) reacting the corresponding cyclic AMP derivative of formula I, wherein R^2 is hydrogen, or a base addition salt thereof, with an acylating agent derived structurally from an acid of the formula R^3O . CO . OH, R^4NH . CO . OH or R^5 . CO . OH, or with an isocyanate of the formula R^4 . N=C=O, wherein R^3, R^4 and R^5 are as stated in claim 1, or

b) reacting the corresponding cyclic AMP derivative of formula I, wherein R^1 is hydrogen and R^3 is an acyl group of the formula —CO . OR^3, —CO . NHR^4 or —CO . R^5, or a base addition salt thereof, with an acylating agent derived structurally from an acid of the formula R^3O . CO . OH or R^4NH . CO . OH, or with an isocyanate of the formula R^4 . N=C=O, wherein R^3 and R^4 are as stated in claim 1;

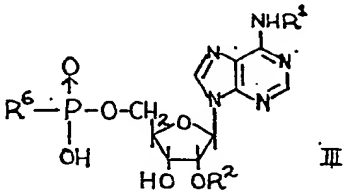
5) dehydrating an adenosine - 5′ - phosphate derivative of the formula: —



II

wherein R^1 and R^2 are as stated in claim 1; or

6) reacting a compound of the formula: —



III

wherein R^1 and R^2 are as stated in claim 1 and R^6 is a radical derived from an acid R^6H which is more acidic than the 3′ - hydroxyl radical of the nucleotide of formula III, with a base.

11. A pharmaceutical composition which comprises a cyclic AMP derivative as claimed in claim 1 in a sterile injectable medium.

12. A composition as claimed in claim 11 which is a sterile injectable solution.

13. A cyclic AMP derivative as claimed in claim 1 substantially as described in any one of Examples 1 to 19.

14. A cyclic AMP derivative as claimed in claim 1 substantially as described in any one of Examples 20 to 34.

A. H. LAIRD,
Agent for the Applicants.

Printed for Her Majesty's Stationery Office, by the Courier Press, Leamington Spa, 1975.
Published by The Patent Office, 25 Southampton Buildings, London, WC2A 1AY, from which copies may be obtained.